# Induction and termination of embryonic diapause in the salt marsh mosquito, *Aedes sollicitans* (Diptera: Culicidae)

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# FOREWORD

About 14,800 acres of salt marsh exist along some 144 miles of Connecticut coastline. These marshes are not only highly efficient producers of plant organic matter, but are also contributors of substantial numbers of noxious insects belonging to the order Diptera. Two recent Station publications have related information on two of these groups of Diptera: nonbiting midges (Frontiers of Plant Science, Fall 1966), and deer flies (Frontiers of Plant Science, Fall 1969). Other Diptera include the greenheads or horseflies, the punkies or no-see-ums, the stable fly, and the salt marsh mosquitoes. This bulletin is concerned with the salt marsh mosquito, *Aedes sollicitans*.

The importance of *A. sollicitans* stems from its biting habits, its ability to function as a vector of the virus causing Eastern Equine Encephalitis, and its role as an intermediate host of the nematode or roundworm, *Dirofilaria immitis,* causing dog heartworm. Although it is one of the most important mosquitoes in North America, much of its basic biology is incompletely understood. This article emphasizes the role of photoperiod and temperature in regulating the seasonal presence of *A. sollicitans* in Connecticut.

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## Introduction

Mosquitoes overwinter as fertilized females, fully formed embryos within egg shells or as late instar larvae. Photoperiod has been shown to control in part the seasonal cycles of multivoltine species which overwinter in these stages of growth (see for example Baker, 1935; Love and Whelchel, 1955; Vindogradova, 1960 and 1965; Depner and Harwood, 1966; Eldridge, 1966; Kappus and Venard, 1967; Anderson, 1968). Very little effort, however, has been directed towards studying the effect of photoperiod on the hatching of embryos of floodwater mosquitoes; that is, those species which lay their eggs on soil and overwinter as fully formed embroyos within egg shells. This paper examines the influence of photoperiod and temperature on the induction and termination of embryonic diapause in *Aedes sollicitans* (Walker), possibly the most important mosquito in Connecticut and one of the more important ones in North America.

*Aedes sollicitans* is primarily a brackish water mosquito occurring along the Atlantic Coast from New Brunswick, Canada to Florida and the British West Indies and westward along the Gulf of Mexico to Texas and Mexico (Howard *et al.,* 1917; and Carpenter and LaCasse, 1955). Interestingly enough, it is also an important species in localized areas of the midwest where unusually high concentrations of sulfur have accumulated in the soil (Horsfall, 1955) or where salt water is present (Fellton, 1944). Knight (1967) has concluded that the average minimal temperature limiting its northward spread is —30°F.

Two or more broods per year are commonly produced throughout its range. Larvae are found from April or May to October along the north Atlantic seaboard in the United States (Carpenter and LaCasse, 1955), and in the extreme south, breeding is continuous throughout the year (Carpenter and Chamberlain, 1946; McGregor and Eads, 1943; King, *et al.,* 1944; Griffitts, 1928). Hatching in New Jersey has been recorded in March by Smith (1904b), though Headlee (1931) reports that this early hatching is scattered and is of no economic importance. Noticeable numbers begin to hatch about May 1 in southern New Jersey, but large numbers usually do not hatch in New Jersey or Connecticut until July (Headlee, 1931; Wallis, 1960). Considerable numbers often are present through September but very few are still on the wing in late October (Headlee, 1931; Britton, 1934; Wallis, 1960).

The specific number of broods a year is determined partly by rainfall and the height and frequency of tidal waters (Smith, 1904a). Six to eight broods a year commonly arise from the salt marshes in New Jersey. Britton and Viereck (1905) observed 7 broods during 1904 in Connecticut.

## Methods

Two strains of *A. sollicitans* were studied. The northern one was isolated from a wild population in Milford, Connecticut (41° 51′ north latitude) during the summer of 1967. This strain has been maintained continuously in the laboratory since that time. The southern strain was isolated from a population in the vicinity of Vero Beach, Florida (27° 15′ north latitude) in 1969 and the $F_2$ and $F_3$ generations of the original isolates utilized.

All rearing was done in metal cans with photoperiod control as described by Anderson (1968). Larval rearing was done in plastic boxes covered with a layer of Saran Wrap®. The rearing medium consisted of 75cc of distilled water, 0.04 g of live granulated yeast and a small amount of wood chip debris. An additional 0.08 g of yeast was added to the medium prior to pupation. The rearing medium was changed whenever scum formed at the surface. Twenty-five larvae were placed in each container and survival averaged more than 90%.

Upon completion of larval development, pupae were transferred to plastic vessels containing clean tap water. These vessels were placed in pint cardboard containers covered with netting. Adults were mated about 5 days following emergence by the method described by McDaniel and Horsfall (1957). A Schuco vacuum® with a pen-like holder and a blunt, bent needle was found to be useful for holding the female in the mating postion (Fig. 1). Adults were fed human blood following mating.

Eggs were handled and subjected to the hatching stimulus (dilute solution of nutrient broth) in essentially the same manner that was described for *A. atropalpus* (Anderson, 1968). Eggs were either submerged in the hatching medium shortly after the completion of embryogeny or submerged following an interval of storage under specific temperature and light conditions. In the former experiments, embryos were 6 days old at the time of submergence when


Figure 1. Mating *A. sollicitans* via the McDaniel-Horsfall technique and demonstrating the Schuco® vacuum pen-like holder with a blunt, bent needle.

the incubation temperature was 32°C; 10 days old when the incubation temperature was 23°C; and 13 days old when the incubation temperature was 18°C. All hatching was carried out at 23°C.

In experiments where the effect of storage of fully formed embryos on hatching was evaluated, eggs were handled in the following manner. A pool of recently deposited eggs obtained during a two to three day period from many females was subdivided into lots containing 50-75 eggs. Lots were kept in a moist container covered with Saran Wrap® and at periodic intervals following deposition, lots were removed and placed in the hatching medium. Eggs were submerged only once, and a different lot was used at each interval.

The criterion used to determine the developmental state of fully formed embryos of *A. sollicitans* was their ability to respond to the hatching stimulus. Embryos that hatched were considered as nondiapausing while those that did not were categorized as diapausing. Unhatched eggs were dechorionated in a solution of sodium hypochlorite to determine their viability. Only fully formed, whitish embryos were considered viable.

Data in Tables 1-4 were transformed by the inverse sine transformation (Steel and Torrie, 1960) before performing the analysis of variance.

## Combined effect of temperature and photoperiod on induction of diapause

Groups of the Connecticut strain of *A. sollicitans* were maintained from the time of hatching of the parent generation to the time of completion of embryonic development of the $F_1$ progeny under a short-day photoperiod [light: dark (L:D) hourly ratio per 24 hour cycle] of 11:13 or a long-day photoperiod (15:9), and low (18°C) or high (32°C) temperatures. Photoperiods selected were those representing conditions near the extremes which the species encounters during its early summer and fall development.

Results presented in Table 1 indicate that photoperiod has a very marked influence on the initiation of diapause. More than 95% of the embryos hatched when the photoperiod was 15:9 at both the high and low temperatures. Under

**Table 1. The combined effect of temperature and photoperiod on the incidence of embryonic diapause in the Connecticut strain of *Aedes sollicitans*.**

| Photoperiod L:D | Temperature °C | Number replicates | Total number Viable eggs | Total number Hatched eggs | Mean % embryonic hatch among replicates* |
|---|---|---|---|---|---|
| 11:13 | 18 | 3 | 827 | 2 | 0.04a |
| | 32 | 3 | 404 | 279 | 70.71 b |
| 15:9 | 18 | 5 | 814 | 783 | 96.60 c |
| | 32 | 3 | 510 | 509 | 99.10 c |

* Treatments having common letter not significantly different at 1% level.

Duncan's multiple range test (Duncan, 1955).

the short-day photoperiod, virtually none of the eggs hatched at the low temperature. At the high temperature and under an L:D of 11:13, 71% of the embryos hatched.

Based on studies with other insects, it is well known that the maximum effect of short days on initiation of diapause is often revealed only within a given temperature range. *A. sollicitans* proved to be no exception. The high temperature partially masked the influence of the short-day photoperiod as it did with *A. atropalpus* (Anderson, 1968). This is of no ecological significance because such temperatures would be most likely to occur only for a few hours of each day in mid-summer when the length of day is favorable for continuous growth.

The independence of the effect of long-day photoperiods from that of low temperature on hatching emphasizes that the deposition of diapausing eggs during autumn is clearly an expression of the influence of shorter days on the mosquito.

## Range of photoperiods inducing diapause

The finding that diapause can be induced by a photoperiod of 11:13 raised the question as to the range of photoperiods capable of inducing embryonic diapause in the Connecticut population. Mosquitoes were, therefore, kept at a medium temperature of 23°C and light periods ranging from 1 to 24 hours per day.

As shown in Table 2, the critical daylength was between 13 to 14 hours. Light periods of 14 hours or more per day resulted in hatching rates of 90% or higher. Periods of light of 8 through 13 hours were effective in inducing diapause in almost all embryos. Twenty-two percent of the embryos hatched under a photoperiod of 6:18, and 75% hatched at a photoperiod of 4:20. The percentage hatching at the very short-day photoperiods was not significantly different from that hatching at 14 hours of light or more.

The short-day, long-day diapause induction curve (Beck, 1968) that could be drawn from data shown in Table 2 was similar to that found for *Aedes atropalpus* (Anderson, 1968). Perhaps this type of induction curve will be commonly encountered in *Aedes* species inhabiting temperate climates. Not all mosquitoes react similarly, however. *Wyeomyia smithii*, the pitcher plant mosquito, from Connecticut, has a long-day induction curve. The critical photoperiod is between 14:10 and 15:9, and all photoperiods with shorter light periods result in diapausing larvae (Anderson, unpublished).

The Florida strain of *A. sollicitans* was taken from a population 14 degrees of latitude south of Connecticut. This strain was reared at 23°C and maintained under photoperiods ranging from 9:15 to 16:8. Data shown in Table 3 demonstrate that diapause is not induced by short-day photoperiods at 23°C in this southern group of mosquitoes.

The response of the Florida strain to photoperiod was expected. Provost (1951) reports *A. sollicitans* as a winter and spring mosquito, though light trap collections suggest that it is most abundant in the summer (Provost, unpublished). Other authors (Carpenter and Chamberlain 1946; McGregor and Eads, 1943; King *et al.* 1944; Griffitts, 1928) have reported the occurrence of larvae throughout the year in southern latitudes. It is, of course, possible

Table 2. The effect of photoperiod on the incidence of embryonic diapause in the Connecticut strain of *Aedes sollicitans* reared at 23°C.

| Photoperiod L:D | Number replicates | Total number Viable eggs | Hatched eggs | Mean % embryonic hatch among replicates* |
|---|---|---|---|---|
| 1:23 | 3 | 533 | 497 | 94.92a |
| 2:22 | 3 | 1685 | 1554 | 91.56a |
| 4:20 | 3 | 1594 | 1049 | 75.04ab |
| 6:18 | 3 | 1111 | 268 | 22.44 bc |
| 8:16 | 3 | 1446 | 2 | .12 c |
| 10:14 | 3 | 1823 | 14 | .95 c |
| 11:13 | 3 | 1265 | 1 | .85 c |
| 12:12 | 3 | 687 | 0 | 0 c |
| 13:11 | 3 | 627 | 0 | 0 c |
| 14:10 | 3 | 802 | 753 | 92.54a |
| 15:9 | 3 | 1434 | 1433 | 99.58a |
| 16:8 | 3 | 1526 | 1462 | 97.22a |
| 20:4 | 3 | 3472 | 3386 | 97.52a |
| 24:0 | 2 | 2111 | 2073 | 97.94a |

* Treatments having common letter not significantly different at 5% level. Duncan's multiple range test (Duncan, 1955).

that a short-day photoperiod response might be noted at temperatures lower than 23°C.

## Stages of development sensitive to photoperiod

To determine the stages of growth sensitive to photoperiod, groups of mosquitoes of the Connecticut strain were reared at 23°C and transferred from a long-day environment to a short-day one, and vice versa during specific stages of development as shown in Table 4.

This series of experiments demonstrates that the effect of photoperiod is cumulative, beginning during the late larval instars. Diapausing embryos develop when mosquitoes are kept continuously at a photoperiod of 10:14 (Table 2). Mosquitoes transferred from a long-day to a short-day photoperiod most approximated this condition when the transfer occurred at the beginning of the third instar (Table 4). Significantly higher rates of hatching occurred when all four larval instars were exposed to the long-day photoperiod. A much higher hatch resulted when the pupal stage was also kept at the long-day photoperiod. When embryos alone were exposed to the 10 hours of light per day, the percentage hatch was lower but not significantly so compared to the 96% hatch obtained when just the initial two instars were kept at an L:D of 10:14.

Table 3. The effect of photoperiod on the incidence of embryonic diapause in the Florida strain of *Aedes sollicitans* reared at 23°C.

| Photoperiod L:D | Number replicates | Total number Viable eggs | Total number Hatched eggs | Mean % embryonic hatch among replicates* |
|---|---|---|---|---|
| 9:15 | 2 | 1378 | 1335 | 96.62 |
| 10:14 | 3 | 1692 | 1580 | 91.19 |
| 11:13 | 3 | 1809 | 1712 | 89.74 |
| 12:12 | 2 | 910 | 888 | 97.57 |
| 13:11 | 2 | 820 | 797 | 97.09 |
| 14:10 | 2 | 1742 | 1701 | 96.16 |
| 15:9 | 2 | 1786 | 1749 | 97.40 |
| 16:8 | 2 | 1709 | 1696 | 99.11 |

* None of the treatments was significantly different from the others.

Table 4. The effect of long-day and short-day photoperiods applied to given combinations of development on induction of embryonic diapause in the Connecticut strain of A. *sollicitans* reared at 23°C.

| Stages of growth exposed to specific photoperiods L:D—15:9 | Stages of growth exposed to specific photoperiods L:D—10:14 | Number replicates | Total number Viable eggs | Total number Hatched eggs | Mean % embryonic hatch among replicates* |
|---|---|---|---|---|---|
| 1, 2** | 3, 4, P, A, E | 2 | 1578 | 36 | 2.52 e |
| 1, 2, 3 | 4, P, A, E | 3 | 1117 | 65 | 9.52 de |
| 1, 2, 3, 4 | P, A, E | 3 | 1924 | 704 | 34.31 cd |
| 1, 2, 3, 4, P | A, E | 2 | 834 | 585 | 70.09ab |
| 1, 2, 3, 4, P, A | E | 3 | 1543 | 1214 | 79.38a |
| 3, 4, P, A, E | 1, 2 | 2 | 1425 | 1378 | 96.75a |
| 4, P, A, E | 1, 2, 3 | 1 | 680 | 648 | 95.29a |
| P, A, E | 1, 2, 3, 4 | 4 | 2591 | 2140 | 80.39a |
| A, E | 1, 2, 3, 4, P | 2 | 575 | 466 | 82.69a |
| E | 1, 2, 3, 4, P, A | 5 | 1997 | 1069 | 43.43 bc |

* Treatments having common letter not significantly different at 5% level.

Duncan's multiple range test (Duncan, 1955).

** Numbers indicate larval instars; P = pupa; A = adult; E = $F_1$ embryo.

The developing embryo was shown to be quite sensitive in the reverse experiments (Table 4). A mean percentage hatch of 43% was obtained when just the embryo was exposed to the long-day photoperiod. A significantly higher percentage of embryos hatched when the pupa and adult were also exposed to the long-day photoperiod. The percentage hatching when the pupa, adult and embryo were kept at 15:9 was lower, but not significantly, than when the fourth larval instar was also maintained under the same conditions.

The late larval instars and subsequent stages of growth, including the developing $F_1$ embryo, are sensitive to length of day. The sensitive stages in *A. sollicitans* are not clearly as discernible as those in *A. atropalpus* where the fourth larval instar and pupa are sensitive (Anderson, 1968). The developing $F_1$ embryo has been shown to be the sensitive stage in *A. triseriatus* (Kappus and Venard, 1967).

## Sensitivity of fully formed embryos to photoperiod

The deposition of eggs that retain their viability out of water for months during all seasons of the year is a characteristic of *A. sollicitans* and other floodwater mosquitoes. *A. sollicitans* is most abundant in July through September in New Jersey (Headlee, 1931). Broods commonly hatch in October (Smith, 1907 and 1908; Howard *et al.,* 1917) but embryos seldom hatch in November with the exception of a small brood in southern New Jersey (Headlee, 1921). The lower percentage or complete lack of hatching in October and November in northern United States may be due in part to the influence of shorter days and cooler temperatures on the fully formed embryos.

To determine the effect of photoperiod on fully formed embryos, groups of mosquitoes were kept at photoperiods of 15:9 or 10:14 at 23°C throughout the larval, pupal and adult stages. Embryos were maintained at their respective photoperiods throughout the first 10 days of embryonic life, and were then either kept at that same photoperiod or transferred to the other one. Eggs were submerged in the hatching medium at weekly intervals.

Embryos maintained continuously at a photoperiod of 10:14 remained in a diapausing state throughout the duration of the experiment (Fig. 2). Hatching of less than 5% was noted 10 days as well as 87 days after deposition. These data suggest that mosquitoes hatching in autumn when the length of day is less than 14 hours (Table 2), will develop and subsequently deposit diapausing eggs that will not hatch that same calendar year regardless of the number of times the eggs are inundated.

Embryos maintained continuously at a photoperiod of 15:9 remained in a non-diapausing state for about 1 month, but then a decrease in hatching ensued (Fig. 3). Mean hatching dropped to 62% when embryos were 59 days old, but then rose slightly. Reduction in percentage hatch of eggs following storage for a number of days has been reported in *A. sollicitans* collected in Delaware (Connell, 1941) and Florida (Travis, 1953), and in other floodwater species such as *A. taeniorhynchus* (Travis, 1953) and *Psorophora varipes* (Aboualy and Horsfall, 1968). These data indicate that eggs laid in the early part of summer will practically all remain in a nondiapausing state for up to a month. After one month, the majority of embryos will hatch so long as daylength is 14 or more hours per day and the temperature is near 23°C, but the percentage of embryos hatching will be less than if they had been flooded at an earlier age.

Embryos transferred from a long-day photoperiod to a short-day one showed an abrupt decrease in hatching after 14 days of storage at an L:D of 10:14 (Fig. 3).. Only about 10% of the embryos hatched when they were 38 days old, but the percentage rose thereafter until almost 50% were hatching when embryos were 87 days old. However, the sharp drop in the number hatching at 24 days indicated that the fully developed embryo is in fact sensitive to photoperiod.

To determine if long-day photoperiods alone could reactivate diapausing embryos, 10 day old embryos were transferred to a 15:9 photoperiod at 23°C and groups of eggs exposed to the hatching medium at weekly or biweekly intervals thereafter. Results shown in Fig. 2 demonstrate, like the data shown in Fig. 3, that fully formed embryos are sensitive to photoperiod. Diapause in 30 to 50% or more of the embryos can be terminated by exposure to long-day photoperiods and temperatures of 23°C for 14 or more days.

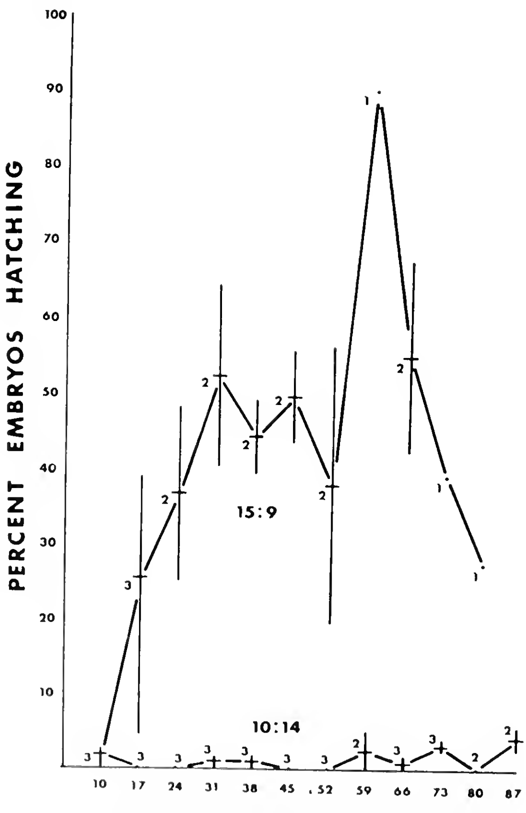


Figure 2. The effect of storage of embryos of the Connecticut strain of *Aedes sollicitans* at 23°C and photoperiods of 10:14 or 15:9 on hatching. Parents and developing embryos (initial 10 days of embryogeny) were maintained at 23°C and an L:D of 10:14. The vertical lines represent observed ranges and the short crosslines are the means, connected by a solid line. The numbers to the left of the means are the number of replicates.

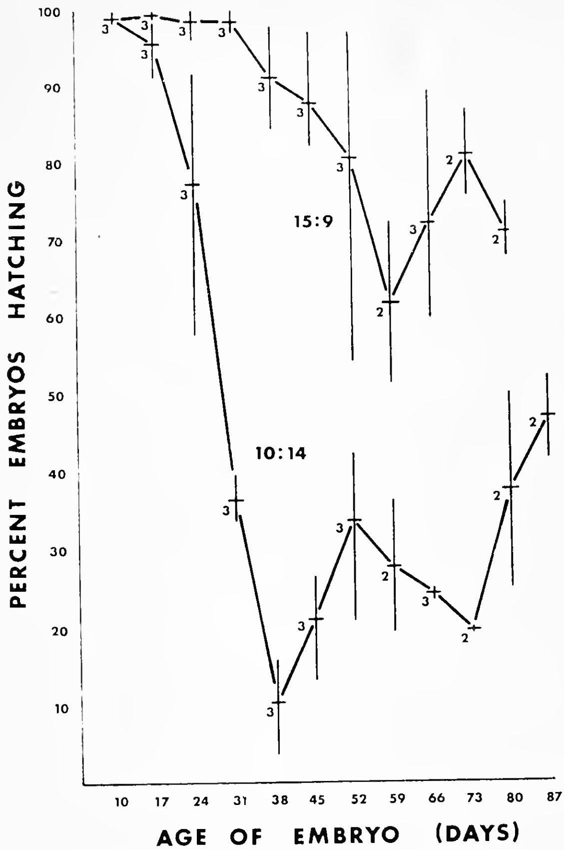


Figure 3. The effect of storage of embryos of the Connecticut strain of *Aedes sollicitans* at 23°C and photoperiods of 10:14 or 15:9 on hatching. Parents and developing embryos (initial 10 days of embryogeny) were maintained at 23°C and an L:D of 15:9. The vertical lines represent observed ranges and the short crosslines are the means, connected by a solid line. The numbers to the left of the means are the number of replicates.

## Sensitivity of fully formed embryos to photoperiod and cool temperatures

Cooler temperatures accompany photoperiods with shorter days in autumn. Table 5 shows the minimum-maximum temperatures in a pool in Milford, Connecticut, from October 9, 1968 to April 2, 1969. Summer temperatures were not recorded, but Connell (1940) reports that mean temperatures in pools in Delaware from June 13 to September 9 ranged from 25°C to 27°C. Lake (1966) reported similar findings in a later study. Temperatures averaged near 23°C for the first part of July and were slightly higher during the first half of August. Temperatures in Connecticut will average close to those reported in Delaware.

A series of experiments was run to determine the effect of storage of fully formed embryos at cool temperatures and long-day and short-day photoperiods on hatching. Mosquitoes were reared at 23°C under a photoperiod of 15:9. When embryos were 10 days old, they were divided into two groups which were further subdivided into lots of 50-75 eggs and transferred to 16°C where the photoperiod was either 10:14 or 15:9. Lots were submerged in the hatching medium at weekly intervals thereafter.

A gradual decline in hatching was noted in embryos maintained continuously at an L:D of 15:9 (Fig. 4). The decline began when embryos were 31 days old and continued until the experiment was terminated. By the time embryos were 80 days old, less than 10% hatched when subjected to the hatching stimulus. Prolonged storage at cool temperatures under long-day photoperiods leads to initiation of diapause.

When embryos were transferred to the short-day photoperiod at 16°C, a decrease in hatching was noted immediately. A sharp decline occurred between the third and fourth week after transfer. Less than 10% hatched when embryos were 52 days old and none hatched when embryos were 73 days old. The abrupt decline in hatching after embryos were one month old suggests that short-day photoperiods and lower temperatures are primarily responsible for the lack of hatching of summer deposited eggs in late autumn in Connecticut.

The effect of short-day photoperiods on mosquitoes developing in late September and October (Tables 2 and 4 and Fig. 2), and the effect of short-day photoperiods and cool temperatures on the fully formed embryo (Fig. 5) account for the decrease or absence of hatching in late October and early November at a north latitude of 41°.

**Table 5. Maximum-minimum temperatures in a salt marsh pool in Milford, Connecticut, 1968-69.**

| Date | Maximum | Minimum |
|---|---|---|
| Oct 9 - 16 | 21 | 10 |
| Oct 16 - 23 | 24 | 12 |
| Oct 23 - 30 | 19 | 7 |
| Oct 30 - Nov 6 | 16 | 1 |
| Nov 6 - 13 | 12 | 4 |
| Nov 13 - 20 | 11 | 8 |
| Nov 20 - 27 | 11 | 7 |
| Nov 27 - Dec 5 | 11 | 9 |
| Dec 5 - 11 | 6 | 0 |
| Dec 11 - 18 | Ice covered pool | |
| Dec 18 - 26 | Ice covered pool | |
| Dec 26 - Jan 2, 1969 | Ice covered pool | |
| Jan 2 - Apr 2 | 12 | 0 |

The data fit well with the field observation that the largest numbers of *A. sollicitans* often emerge in July through September. Medium temperatures and day lengths conducive for continuous growth prevail, at least to the middle of September (the critical daylength is between 13 to 14 hours), and the perigee tides are less than 30 days apart. Heavy rains sometime occur between perigee tides resulting in production of additional broods. Long-day photoperiods, medium temperatures and inundation of fully formed embryos no older than about 30 days are optimum conditions for hatching of *A. sollicitans* in northern United States.

## Endogenous rhythm

Adkisson (1966) has shown in the pink bollworm that once the endocrine mechanism involved in the photoperiodic response is synchronized with a certain

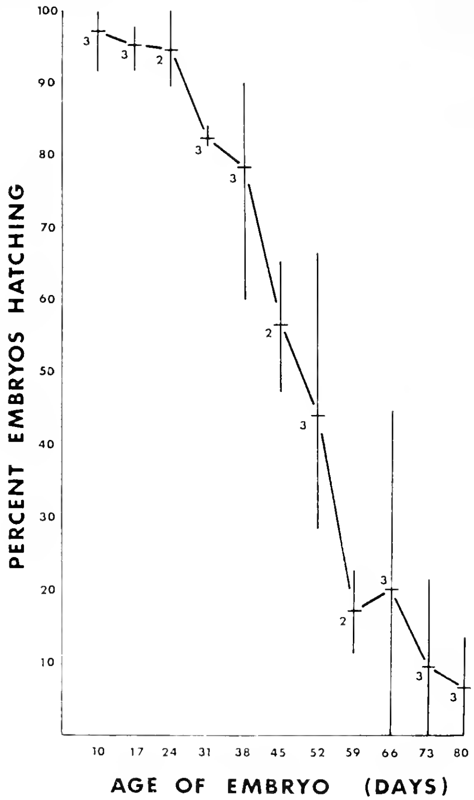


Figure 4. The effect of storage of embryos of the Connecticut strain of *Aedes sollicitans* at 16°C and a photoperiod of 15:9 on hatching. Parents, and developing embryos (initial 10 days of embryogeny) were maintained at 23°C and a photoperiod of 15:9. The vertical lines represent observed ranges and short crosslines are the means, connected by a solid line. The numbers to the left of the means are the number of replicates.

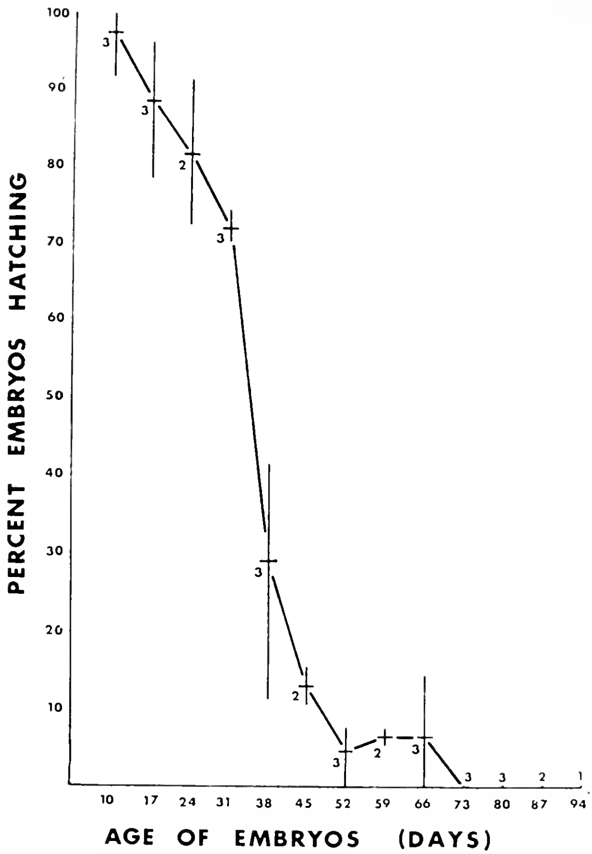


Figure 5. The effect of storage of embryos of the Connecticut strain of *Aedes sollicitans* at 16°C and a photoperiod of 10:14 on hatching. Parents and developing embryos (initial 10 days of embryogeny) were maintained at 23°C and a photoperiod of 15:9. The vertical lines represent observed ranges and the short crosslines are the means, connected by a solid line. The numbers to the left of the means are the number of replicates.

light-dark regime for a period of time, a free running rhythm is established in the absense of temporal cues from the environment. Comparable findings have also been published with the European corn borer (Beck and Alexander, 1964). To determine if a similar rhythmic pattern could be induced in *A. sollicitans*, mosquitoes were initially kept at a photoperiod of 10:14 and then transferred at various stages (beginning of third instar to the initiation of embryonic development) to an aperiodic environment of continuous light. A comparison of results shown in Table 6 with those in Table 4 indicates that such a developmental pattern was not induced.

Short-day photoperiods induce embryonic diapause in *A. sollicitans*. Diapause in some fully formed embryos can be terminated by exposure to a 15:9 photoperiod (Fig. 2). To determine if a free running rhythm could be estab-

**Table 6. The effect of transfer of the Connecticut strain of *Aedes sollicitans* from a short-day photoperiod to an aperiodic environment of continuous light at 23°C on hatching of embryos.**

| Stages of growth exposed to specific photoperiods L:D—10:14 | L:D—24:0 | Total number Viable eggs | Hatched eggs | % hatch |
|---|---|---|---|---|
| 1, 2* | 3, 4, P, A, E | 343 | 331 | 96.5 |
| 1, 2, 3 | 4, P, A, E | 443 | 425 | 95.9 |
| 1, 2, 3, 4 | P, A, E | 1653 | 1602 | 96.9 |
| 1, 2, 3, 4, P | A, E | 289 | 272 | 94.1 |
| 1, 2, 3, 4, P, A | E | 418 | 207 | 49.5 |

* Numbers indicate larval instars; P = pupa; A = adult; E = $F_1$ embryo.

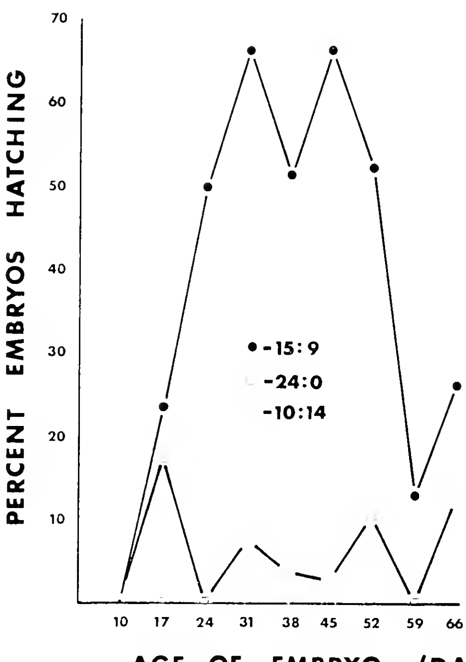


Figure 6. The effect of storage of embryos of the Connecticut strain of *Aedes sollicitans* at 23°C and photoperiods of 10:14, 15:9, and 24:0 on hatching. Parents and developing embryos (initial 10 days of embryogeny) were maintained at 23°C and a photoperiod of 10:14.

lished in fully formed embryos, parents, and embryos for their initial 10 days of life were exposed to a 10:14 photoperiod. Embryos 10 days old were then divided into 3 groups. One group was kept at an L:D of 10:14, another was placed at an L:D of 15:9, and another transferred to continuous light. Results are shown in Fig. 6. Embryos kept continuously at a photoperiod of 10:14 did not hatch. In those transferred to a photoperiod of 15:9, hatching was often in excess of 50% after embryos were 24 days old. These data are in agreement with those shown in Fig. 2. Embryos transferred to the aperiodic environment of continuous light had a hatching pattern similar to, though slightly higher than, those kept continuously under the short-day photoperiod. These data suggest that the developmental pattern established under the influence of the short-day photoperiod was not changed appreciably by the transfer of fully formed embryos to an aperiodic environment.

## Termination of diapause

The terminal seasonal brood of *A. sollicitans* emerges from the salt marsh in October at the latitude of Connecticut (Britton, 1934) and northern New Jersey (Headlee, 1931). The first major seasonal brood hatches during the middle of May in Connecticut (Britton, 1934), and early to mid May in New Jersey (Headlee, 1931) though abundant numbers are not apparent until July (Wallis, 1960; Headlee, 1931). By mid May water temperatures have risen, and photoperiods in excess of 14 hours have been in effect for over one month (List, 1966).

Experiments were carried out to evaluate the effect of cold storage temperatures (5°C) and post-cold photoperiods at 23°C on termination of diapause. Mosquitoes were reared at a photoperiod of 10:14 and the embryos kept under these conditions for the initial 10 days of embryonic development. Embryos were then transferred to a refrigerator at a temperature of 5°C. The light was not controlled and was on only when the refrigerator door was opened. Embryos were removed at bi-weekly intervals after 7 weeks storage and kept at 23°C and a photoperiod of 16:8 for 2 days before placement in the hatching medium. Two replicates were run. The mean percentage hatch at each interval was as follows: Seven weeks of exposure to cold, 89%; 9 weeks of exposure to cold, 82%; 11 weeks of exposure to cold, 94%. These data contrast markedly with those obtained when embryos were kept at an L:D of 10:14 and 23°C for comparable intervals of time (Fig. 2).

To determine if post-cold photoperiods influenced hatching, ten day old diapausing embryos were placed at 5°C and removed at weekly intervals 24 days after initial placement at the cold temperature. Upon removal from the cold, embryos were either placed at a short or a long-day photoperiod for 48 hours prior to placement in the hatching medium. Results shown in Table 7 demonstrate that the post-cold photoperiod is important in determining hatching. Very few embryos hatched following exposure to post-cold treatments of short-day photoperiod. Conversely, most embryos hatched following a post-cold treatment of a long-day photoperiod.

Winter temperatures, post cold photoperiods, and rising water temperatures (Elmore and Fay, 1958) all contribute to termination of diapause. Laboratory studies reported in this section did not simulate all field conditions

**Table 7. The effect of post-cold photoperiods on hatching of embryos of the Connecticut strain of *Aedes sollicitans* following storage at 5°C. Parents, and developing embryos (initial 10 days of embryogeny) were maintained at 23°C and a photoperiod of 10:14.**

| Age of embryo (days) | Days embryos stored at 5°C | Post-cold photoperiod | Total number | | % hatch |
|---|---|---|---|---|---|
| | | | Viable eggs | Hatched eggs | |
| 34 | 24 | 15:9 | 44 | 25 | 56.81 |
| 41 | 31 | 10:14 | 24 | 0 | 0 |
| 48 | 38 | 15:9 | 32 | 23 | 71.87 |
| | | 10:14 | 31 | 1 | 3.22 |
| 55 | 45 | 15:9 | 19 | 14 | 73.67 |
| | | 10:14 | 34 | 3 | 8.82 |
| 62 | 52 | 15:9 | 53 | 48 | 90.56 |
| | | 10:14 | 27 | 1 | 3.70 |
| 67 | 59 | 15:9 | 22 | 19 | 86.36 |
| | | 10:14 | 28 | 4 | 14.28 |

(i.e. photoperiod was not controlled in the refrigerator and low hatching temperatures were not utilized), but the data suggest that these three parameters are important in determining the percentage hatch under natural field conditions. Daylengths of 13½ or more hours occur at this latitude during the latter part of March. Some hatching has been recorded in March (Smith, 1904b), but the first significant hatching does not take place until later in the year. Presumably, medium water temperatures and long-day photoperiods are needed to ensure maximum hatching of overwintering eggs.

## Summary

1. *Aedes sollicitans* is a brackish water mosquito occurring along Long Island Sound in Connecticut. It has several generations each year from May to October. In more southern climates, embryos hatch throughout the year.

2. Photoperiod was shown to control, in large part, embryonic diapause in the Connecticut strain, but not in the Florida one.

3. The Connecticut strain exhibited a long-day, short-day diapause induction curve with the critical photoperiod being positioned between 14:10 and 13:11.

4. The effect of short-day photoperiods on induction of diapause is masked by elevated temperatures.

5. The late larval instars, pupa, adult, developing embryo and fully formed embryo are sensitive to photoperiod.

6. Embryos maintained continuously at a photoperiod of 10:14 at 23°C remained in a diapausing state for 87 days, the duration of the experiment.

7. Embryos maintained continuously at a photoperiod of 15:9 at 23°C remained in a nondiapausing state for about 1 month, but then a decrease of 20-40 percent hatching ensued.

8. Fully formed embryos transferred from a long-day photoperiod to a short-day one at 23°C showed an abrupt decrease in hatching after 14 days storage at an L:D of 10:14. Only about 10% of the embryos hatched when the embryos were 38 days old, but the percentage hatching rose thereafter to about 50% when embryos were 87 days old.

9. Storage of diapausing embryos at long-day photoperiods for 14 days or more at 23°C resulted in termination of diapause in 30-50% of the population.

10. A gradual decline in hatching was observed in embryos maintained continuously at an L:D of 15:9 and stored at 16°C. The decline began when embryos were 31 days old and continued until less than 10 percent were hatching when embryos were 80 days old.

11. A decrease in hatching was observed immediately after embryos were transferred from a long-day to a short-day photoperiod at 16°C. Less than 10% were hatching when embryos were 52 days old.

12. The effect of short-day photoperiods on mosquitoes developing in autumn, and the effect of short-day photoperiods and cool temperatures on fully formed embryos that were deposited during summer account for the decrease or absence of hatching in late autumn in Connecticut.

13. Long-day photoperiods, medium temperatures, and inundation of fully formed embryos no older than 30 days are optimum conditions for hatching of *A. sollicitans* in Connecticut.

14. Data are presented suggesting that an endogenous rhythm can be established in fully formed diapausing embryos.

15. Cold temperatures (5°C) for several weeks, post-cold temperatures of 23°C for two days, and post-cold photoperiods of 15:9 terminate diapause.

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